

***What Is Claimed Is:***

1. A serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of one or more antioxidants, one or more albumins or albumin substitutes, one or more lipid agents, one or more insulins or insulin substitutes, one or more transferrins or transferrin substitutes, one or more trace elements, and one or more glucocorticoids,

wherein a basal cell culture medium supplemented with said supplement is capable of supporting the expansion of CD34<sup>+</sup> hematopoietic cells.

2. A serum-free, eukaryotic cell culture medium supplement comprising one or more antioxidants, and one or more ingredients selected from the group consisting of one or more albumins or albumin substitutes, one or more lipid agents, one or more insulins or insulin substitutes, one or more transferrins or transferrin substitutes, one or more trace elements, and one or more glucocorticoids,

wherein a basal cell culture medium supplemented with said supplement is capable of supporting the expansion of CD34<sup>+</sup> hematopoietic cells.

3. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said antioxidants are selected from the group consisting of N-acetyl-L-cysteine, 2-mercaptoethanol, and D,L-tocopherol acetate, or derivatives or mixtures thereof.

4. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said albumin is human serum albumin.

5. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said lipid agents are selected from the group

consisting of Human Ex-Cite® and ethanolamine or derivatives and mixtures thereof.

6. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said insulin is human zinc insulin.

7. The serum-free, eukaryotic cell culture medium supplement according to claim 1; wherein said transferrin is human iron-saturated transferrin.

8. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said trace element is  $\text{Se}^{4+}$ .

9. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said glucocorticoid is hydrocortisone.

10. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said supplement is concentrated.

11. The serum-free, eukaryotic cell culture medium supplement according to claim 10, wherein said supplement is concentrated about 2 to about 100 fold.

12. The serum-free, eukaryotic cell culture medium supplement according to claim 11, wherein said supplement is about a 40X formulation.

13. A serum-free, eukaryotic cell culture medium supplement obtained by combining one or more ingredients of claim 1.

14. A serum-free, eukaryotic cell culture medium supplement according to claim 13, wherein said ingredients comprise one or more ingredients selected from the group consisting of N-acetyl-L cysteine, human serum albumin,

Human Ex-Cyte®, ethanolamine, human zinc insulin, human iron saturated transferrin,  $\text{Se}^{4+}$ , hydrocortisone, D,L-tocopherol acetate, and 2-mercaptoethanol.

15. A method of making a serum-free eukaryotic cell culture medium supplement, said method comprising admixing water with one or more ingredients according to claim 1.

16. The method according to claim 15, wherein said ingredients comprise water, N-acetyl-L cysteine, human serum albumin, Human Ex-Cyte®, ethanolamine HCl, human zinc insulin, human iron saturated transferrin, a  $\text{Se}^{4+}$  salt, hydrocortisone, D,L-tocopherol acetate, and 2-mercaptoethanol.

17. A kit comprising a carrier means, said carrier means being compartmentalized to receive in close confinement therein one or more container means, wherein a first container means contains the supplement of claim 1, and wherein optionally a second container means contains a basal medium.

18. A serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with the serum-free culture supplement according to claim 1,

wherein said supplemented culture medium is capable of supporting the expansion of  $\text{CD34}^+$  hematopoietic cells.

19. The serum-free eukaryotic cell culture medium according to claim 18, wherein said basal medium is selected from the group consisting of Iscove's Modified Dulbecco's Medium, RPMI-1640,  $\alpha$ -MEM.

20. A serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with the serum-free supplement according to claim 1,

wherein said medium is capable of supporting the expansion of  $\text{CD34}^+$  hematopoietic cells.

21. The serum-free eukaryotic cell culture medium according to claim 20, wherein said medium is a 1X medium.

22. The serum-free eukaryotic cell culture medium according to claim 20, wherein said medium is a concentrated medium formulation.

23. The serum-free eukaryotic cell culture medium according to claim 22, wherein said medium is from about 2X to about 100X concentrated

24. The serum-free eukaryotic cell culture medium according to claim 23, wherein said concentrated medium formulation is about a 10X formulation.

25. The serum-free eukaryotic cell culture medium according to claim 23, wherein said concentrated medium formulation is greater than 10X concentrated.

26. A method of making a serum-free eukaryotic cell culture medium, said method comprising admixing a basal cell culture medium with the serum-free supplement according to claim 1,

wherein said medium is capable of supporting the expansion of CD34<sup>+</sup> hematopoietic cells.

27. A serum-free eukaryotic cell culture medium comprising one or more ingredients selected from the group consisting of one or more antioxidants, one or more albumins or albumin substitutes, one or more lipid agents, one or more insulins or insulin substitutes, one or more transferrins or transferrin substitutes, one or more trace elements, one or more glucocorticoids, one or more inorganic salts, one or more energy sources, one or more buffering agents, one or more pyruvate salts, one or more pH indicators, one or more amino acids, and one or more vitamins,

wherein the medium is capable of supporting the expansion of CD34<sup>+</sup> hematopoietic cells.

28. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said antioxidants are selecting from the group consisting of N-acetyl-L-cysteine, 2-mercaptoethanol, and D,L-tocopherol acetate, or derivatives or mixtures thereof.

29. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said albumin is human serum albumin.

30. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said lipid agents are Human Ex-Cyte<sup>®</sup> and ethanolamine.

31. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said insulin is human zinc insulin.

32. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said transferrin is human iron-saturated transferrin.

33. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said glucocorticoid is hydrocortisone.

34. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, one or more potassium salts, one or more magnesium salts, one or more sodium salts, one or more carbonate salts, and one or more phosphate salts.

35. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said energy source is D-glucose.

36. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said buffering agent is HEPES.

37. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said pyruvate salt is sodium pyruvate.

38. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said pH indicator is phenol red.

39. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said amino acid ingredient comprises one or more amino acids selected from the group consisting of glycine, L-alanine, L-asparagine, L-cysteine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-glutamine, L-arginine HCL, L-methionine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine, and salts and derivatives thereof.

40. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B<sub>12</sub> and derivatives thereof.

41. The serum-free, eukaryotic cell culture medium according to claim 27, wherein said ingredients comprise N-acetyl-L-cysteine, 2-mercaptoethanol, human serum albumin, D,L-tocopherol acetate, Human Ex-Cyte®, ethanolamine, human zinc insulin, iron-saturated transferrin, Se<sup>4+</sup>, hydrocortisone, Ca<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, CO<sub>3</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>, D-glucose, HEPES, sodium pyruvate, phenol red, glycine, L-alanine, L-asparagine, L-cysteine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-glutamine, L-arginine HCL, L-methionine, L-proline, L-hydroxyproline, L-serine, L-threonine,

L-tryptophan, L-tyrosine, and L-valine, biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B<sub>12</sub>.

42. The serum-free, eukaryotic cell culture medium according to claim 41, wherein said medium is obtained by combining water and the ingredients of claim 46.

43. A method of making a serum-free, eukaryotic cell culture medium said method comprising admixing the ingredients according to claim 41.

44. A composition comprising CD34<sup>+</sup> hematopoietic stem cells in the serum-free medium of claim 27.

45. The composition according to claim 44, wherein said CD34<sup>+</sup> hematopoietic stem cells are obtained from an animal selected from the group consisting of human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, and sheep.

46. A method of expanding CD34<sup>+</sup> hematopoietic cells, said method comprising

- (a) contacting said cells with the medium according to claim 27; and
- (b) culturing said cells under conditions suitable to facilitate the expansion of said cells.

47. The method according to claim 46, wherein said culturing further comprises adding a hematopoietic cell growth factor to said medium.

48. The method according to claim 47, wherein said growth factor is one or more growth factors selected from the group consisting of erythropoietin,

granulocyte-colony stimulating factor, stem cell factor, interleukin-3, and granulocyte/macrophage-colony stimulating factor.

49. A method of providing CD34<sup>+</sup> hematopoietic cells, in serum-free culture, to a mammal, said method comprising

- (a) contacting said CD34<sup>+</sup> hematopoietic cells with the medium according to claim 27;
- (b) cultivating said CD34<sup>+</sup> hematopoietic cells under conditions suitable to facilitate the expansion of said cells in serum-free culture; and
- (c) introducing said expanded cells into a mammal.

50. The method according to claim 49, wherein said culturing further comprises adding a hematopoietic cell growth factor to said medium.

51. A method of causing CD34<sup>+</sup> hematopoietic cells to differentiate into a particular type of cell in serum-free culture, said method comprising

- (a) contacting said CD34<sup>+</sup> hematopoietic cells with the medium according to claim 27;
- (b) cultivating said cells under conditions suitable to facilitate the expansion of said cells in serum-free culture; and
- (c) adding one or more differentiation factors or changing culturing conditions to induce differentiation of cells to form a different type of hematopoietic cell.

52. A method of providing differentiated hematopoietic cells, in serum-free culture, to a mammal, said method comprising

- (a) contacting said CD34<sup>+</sup> hematopoietic cells with the medium according to claim 27;
- (b) cultivating said cells under conditions suitable to facilitate the expansion of said cells in serum-free culture;



(c) adding one or more differentiation factors or changing culturing conditions to induce differentiation of cells to form a different type of hematopoietic cell; and

(d) introducing said differentiated cells into a mammal.

53. A method of expanding a recombinant CD34<sup>+</sup> hematopoietic cells, said method comprising

5 (a) obtaining a recombinant CD34<sup>+</sup> hematopoietic cell containing a nucleic acid molecule which encodes a protein of interest; and

(b) culturing said cell in the medium according to claim 27 to form a population of recombinant cells.

54. A method of providing a recombinant CD34<sup>+</sup> hematopoietic cell to a mammal, said method comprising

10 (a) obtaining a recombinant CD34<sup>+</sup> hematopoietic cell containing a nucleic acid molecule which encodes a protein of interest;

(b) culturing said cell in the medium according to claim 27 to form a population of recombinant CD34<sup>+</sup> hematopoietic stem cells; and

15 (c) introducing said recombinant cells into a mammal.